Background and Purpose—Through harmonic gray-scale imaging, it is possible to analyze brain tissue perfusion with different ultrasound methods.

Methods—In 12 healthy volunteers, 2 doses (0.5 and 1.5 mL) of Optison, a perfluoropropane-containing contrast agent, were injected intravenously and produced a strong increase of brightness in the brain parenchyma. We used harmonic imaging for quantification of ultrasound intensity in the thalamus, ipsilateral temporoparietal white matter (TPWM), and ipsilateral lateral fissure at both sides. Time-intensity curves were calculated, and peak increase (PI) of intensity and the area under the time-intensity curve (AUC) from baseline were compared.

Results—We found a significant dose dependence of the AUC in all regions at both sides. PI only showed a significant dose dependence in the TPWM but not in the ipsilateral thalamus and lateral fissure. No side differences for AUC and PI were detected in all regions and doses used. We found a significantly higher value of the PI insonating the thalamus from the ipsilateral side compared with the contralateral side. The same result was obtained for the AUC in the left thalamus for both doses and in the right thalamus for the high dose. Using 0.5 mL for insonation of the right thalamus AUC again showed a higher value for the insonation from the ipsilateral compared with the contralateral side but failed to show statistical significance (P = 0.08, n = 12).

Conclusions—Harmonic gray-scale imaging with Optison showed a strong enhancement effect in the brain parenchyma. A quantitative analysis of perfusion seems difficult because of the depth dependence of the effect. The most robust parameter is the AUC. (Stroke. 2000;31:151-154.)

Key Words: contrast media ♦ perfluorocarbons ♦ perfusion ♦ ultrasonography

Several attempts have been made in the fields of neurosonology and echocardiography to measure capillary blood flow and tissue perfusion by means of contrast-enhanced ultrasound techniques. Harmonic imaging is a new ultrasound method that increases the signal-to-noise ratio in color-coded duplex sonography and gray-scale imaging. It incorporates the phenomenon that gas bubbles of ultrasound contrast agents (UCAs) that can pass the capillary bed of the lungs are highly resonant at frequencies used for diagnostic ultrasound. When a gas bubble vibrates near resonance, it produces harmonics or multiples of the transmitted frequency. Whereas insonated tissues respond primarily at the fundamental frequency, gas-filled UCAs respond at the fundamental and harmonic frequencies. Second harmonic ultrasound systems work to transmit at 1 frequency (fundamental) and receive at twice that frequency (second harmonic). This approach removes the unwanted fundamental frequency (noise), leaving only the second harmonic frequency generated by the UCA. Therefore, the signal-to-noise ratio is dramatically increased by (second) harmonic imaging. We have previously shown that an increased spatial resolution of the vertebrobasilar circulation can be achieved by use of this novel technique in contrast-enhanced color-coded duplex sonography.1 In 2 studies on human healthy volunteers and 1 case report of 2 patients suffering from cerebral infarction, use of second harmonic imaging in gray-scale ultrasound produced signal-enhancing effects in different regions of the brain.2–4 However, the extent of contrast enhancement in the parenchyma was highly variable and depth dependent, probably because of the use of air-based UCAs.

The purpose of this human study was to investigate the contrast-enhancing effects of a new perfluoropropane-based UCA (Optison; Mallinckrodt) and to evaluate harmonic gray-scale imaging with respect to its potential for detection of contrast agent in the microcirculation of the brain. This study is of particular interest for those designing further investigations planning the visualization of perfusion defects in patients with acute ischemic stroke by means of ultrasound methods.
Subjects and Methods

Subjects
Transcranial sonography was performed in 12 healthy subjects (5 women, 7 men; median age, 27.5 years; range, 21 to 44 years; median body mass index, 23.9 kg/m²; range, 20.9 to 32.6 kg/m²) with adequate ultrasonic windows. Exclusion criteria were pregnancy or lactation, a past medical history of cerebrovascular or cardiovascular disease, previous allergic reactions, and abuse of alcohol or substances. A complete physical examination, a 12-lead ECG, and routine blood tests were performed before and 24 hours after administration of UCA. Written, informed consent was obtained from each volunteer before entry into the study. The study was approved by an ethics committee and was carried out in accordance with the guidelines of Good Clinical Practice and the Declaration of Helsinki (1964).

Ultrasound Contrast Agent
Optison (provided by Mallinckrodt Medical GmbH, Hennef, Germany) is a perfluoropropane-containing UCA based on a 1% albumin solution. Octafluoropropane is chemically characterized with a molecular weight of 188 and an empirical formula of C₈F₂₈. The microspheres in Optison injectable suspension are produced by heat treatment and sonication of appropriately diluted human albumin (USP) in the presence of octafluoropropane gas by a proprietary process. The protein in the microsphere shell makes up ~5% to 7% of the total protein in the liquid. Each 1 mL of Optison contains 5.0 to 8.0 × 10⁴ human albumin microspheres, 10 mg albumin human (USP), 0.22 ± 0.11 mg/mL octafluoropropane, 0.2 mg N-acetylcysteine, and 0.12 mg caprylic acid in 0.9% aqueous sodium chloride. The headspace of the vial is filled with octafluoropropane gas. The pH is adjusted with sodium hydroxide to 6.4 to 7.4. The microsphere particle size parameters are mean diameter (range) 2.0 to 4.5 μm (maximum, 32.0 μm); 93% less than 10 μm. Octafluoropropane is a stable gas that is not metabolized. The human albumin component of the microsphere is expected to be handled by the normal metabolic routes for human albumin. The UCA is commercially available and was originally developed for echocardiography (Generic FS069, Mallinckrodt Inc.). The solution was prepared following the manufacturer’s instructions. Two intravenous bolus injections of 0.5 and 1.5 mL (injection speed, 1 mL/s) were used. Each injection was followed immediately by a second bolus of 3 mL of 0.9% NaCl solution to ensure clearance of the residual UCA in the venous system. The time between 2 UCA bolus injections was 5 to 10 minutes.

Transcranial Sonography
Harmonic gray-scale imaging was performed with an HP SONOS 5500 ultrasound system (Hewlett Packard) connected to a 1.8/3.6-MHz sector transducer (S4 probe, Hewlett Packard) in an investigation depth of 10 cm (focus on 8 cm). For gray-scale imaging, we used the integrated backscatter (IBS) mode and the study type T-INT (mechanical index, 1.0 to 1.1).

After each UCA injection, 62 digitized gray-scale images of the brain triggered by ECG were stored in continuous-loop-review memory and then recorded on an optical disk for later offline analysis. We used the transient response imaging mode with a frame rate of 1 image every 4 cardiac cycles. Gain and transmit power settings were optimized for each volunteer at the beginning of each investigation and were not changed throughout the procedure. The entire investigation was also recorded on videotape.

Harmonic Gray-Scale Imaging
For analysis of harmonic gray-scale data, the IBS of brain tissue was measured offline with the acoustic densitometry unit of the HP SONOS 5500. This unit assists in the quantification of ultrasound images by measuring the scattered energy received by the transducer. Because acoustic densitometry measurement is made upstream in the imaging chain, it is less influenced by postprocessing functions of the imaging chain. The IBS is a relative measure of the total ultrasound energy scattered by a small volume of the interrogated tissue. The IBS data measurement were displayed on a logarithmic scale in decibels. We specified the regions of interest (ROIs) to the thalamus region at both sides, ipsilateral temporoparietal white matter, and ipsilateral lateral fissure, where branches of the middle cerebral artery are located.

Identification of the anatomic sites has been described previously. The thalamus regions and parts of the lateral fissure, where branches of the middle cerebral artery are located. Identification of the anatomic sites has been described previously. The sample volume of the ROIs was 21×21 pixels. The mean IBS in the ROIs of the first 2 images served as baseline reference (noise floor). The change in the IBS in the seconds after UCA injection was measured, and the mean values were displayed graphically.

In this study, we compared the area under curve (AUC) and the peak increase (PI) from baseline in the brain parenchyma (third ventricle, pineal gland) for increasing doses of Optison using a nonparametric test for related samples (Friedman ANOVA test). Baseline for each ROI was the mean value from the first 2 acoustic densitometry values after injection.

Results
Figure 1 shows the insonation plane used to insonate the thalamus regions and parts of the lateral fissure, where branches of the middle cerebral artery are located. After bolus injections of 0.5 and 1.5 mL, we observed a strong increase in brightness.
of the brain parenchyma on both sides in all volunteers. When the washout curves after 0.5- and 1.5-mL bolus injections of Optison were analyzed and the mean washout curves in the 3 ROIs were plotted from the 12 individuals under investigation, we again observed only minor side differences in the IBS–heart cycle curves. We analyzed 2 parameters of the washout curves (PI and AUC) with respect to their dose dependence (Table 1) and side differences for the different doses. For the 3 ROIs (ipsilateral thalamus; lateral fissure, where branches of the middle cerebral artery are located; and temporoparietal white matter), we found a significant dose dependence for the AUC in all regions and for the PI in only the temporoparietal white matter but not in the ipsilateral thalamus and lateral fissure (Table 1). We detected no side difference for AUC and PI in all regions and doses used ($P<0.13$ to 0.76, $n=12$).

With a maximum insonation depth of 10 cm, we could image the ipsilateral and contralateral thalamus. In the healthy young volunteers under examination, we assume similar perfusion in the thalamus at both sides. In Figure 2, washout curves for the 2 doses of Optison in the ipsilateral and contralateral thalamus are displayed to determine the depth dependence of UCA detection and of the parameter characterizing the washout curves (PI and AUC).

For 0.5 and 1.5 mL, we found a significantly higher value of the PI insonating the thalamus from the ipsilateral compared with contralateral side (Table 2). We found the same result for the AUC in the left thalamus for both doses and in the right thalamus for the high dose. With 0.5 mL used for insonation of the right thalamus, the AUC again showed a higher value for the insonation from the ipsilateral compared with contralateral side but failed to show statistical significance ($P=0.08$, $n=12$).

Ultrasound examinations and injections of UCAs were generally well tolerated. One subject reported a mild, transient headache after the investigation; another volunteer experienced a mild burning sensation in the right upper abdominal quadrant that resolved completely within an hour. No abnormalities in physical state or blood chemical values were noted on follow-up examination.

### Discussion

With gray-scale imaging, a homogenous, dose-dependent increase in the brightness of the brain parenchyma was detected. In our opinion, the most robust parameter for the description of the enhancing effects is the intensity–heart cycle AUC, which showed a significant dose-dependent

### Table 1. Regional Differences and Dose Dependence of the Enhancing Effects of Optison

<table>
<thead>
<tr>
<th>Structure</th>
<th>Side</th>
<th>Dose, mL</th>
<th>PI, dB</th>
<th>$P^*$</th>
<th>AUC, dB×cc</th>
<th>$P^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>THAL, ipsi</td>
<td>Right</td>
<td>0.5</td>
<td>9.6±3.1</td>
<td>0.15</td>
<td>674.7±173.4</td>
<td>0.0005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>11.5±1.9</td>
<td></td>
<td>1153.2±264.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>0.5</td>
<td>11.0±3.5</td>
<td>0.13</td>
<td>844.4±459.8</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>11.7±2.2</td>
<td></td>
<td>1177.0±402.9</td>
<td></td>
</tr>
<tr>
<td>MCA</td>
<td>Right</td>
<td>0.5</td>
<td>14.3±4.1</td>
<td>0.08</td>
<td>1263.3±514.0</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>15.2±4.1</td>
<td></td>
<td>1894.1±617.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>0.5</td>
<td>15.4±2.8</td>
<td>0.13</td>
<td>1428.9±477.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>16.0±3.7</td>
<td></td>
<td>2023.5±814.3</td>
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<tr>
<td>TPWM</td>
<td>Right</td>
<td>0.5</td>
<td>12.6±2.8</td>
<td>0.04</td>
<td>727.4±267.2</td>
<td>0.0005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>14.3±3.6</td>
<td></td>
<td>1379.1±505.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>0.5</td>
<td>13.6±3.1</td>
<td>0.0009</td>
<td>889.5±404.7</td>
<td>0.0009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>15.9±4.5</td>
<td></td>
<td>1460.6±547.7</td>
<td></td>
</tr>
</tbody>
</table>

cc indicates cardiac cycles; THAL, ipsi, ipsilateral thalamus; MCA, lateral fissure where branches of middle cerebral artery MCA are located; and TPWM, temporo-parietal white matter.

*Friedman ANOVA test.

### Table 2. Depth Dependence of the Enhancing Effects of Optison

<table>
<thead>
<tr>
<th>Site</th>
<th>Dose, mL</th>
<th>Region</th>
<th>PI, dB</th>
<th>$P^*$</th>
<th>AUC, dB×cc</th>
<th>$P^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right THAL</td>
<td>0.5</td>
<td>Ipsilateral</td>
<td>9.6±3.1</td>
<td>0.02</td>
<td>674.7±173.4</td>
<td>0.08</td>
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<tr>
<td></td>
<td></td>
<td>Contralateral</td>
<td>8.1±2.0</td>
<td></td>
<td>505.9±206.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>Ipsilateral</td>
<td>11.5±1.9</td>
<td>0.009</td>
<td>1153.2±264.6</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Contralateral</td>
<td>8.8±2.2</td>
<td></td>
<td>887.6±363.2</td>
<td></td>
</tr>
<tr>
<td>Left THAL</td>
<td>0.5</td>
<td>Ipsilateral</td>
<td>11.0±3.5</td>
<td>0.001</td>
<td>844.4±459.8</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Contralateral</td>
<td>7.9±3.4</td>
<td></td>
<td>525.2±356.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>Ipsilateral</td>
<td>11.7±2.2</td>
<td>0.001</td>
<td>1177.0±402.9</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Contralateral</td>
<td>8.1±2.0</td>
<td></td>
<td>766.8±319.7</td>
<td></td>
</tr>
</tbody>
</table>

THAL indicates thalamus; ipsilateral, insonation depth of 7 cm; contralateral, insonation depth of 8.5 cm; and cc, cardiac cycles.

*Friedman ANOVA test.
increase and no side differences. With the dye dilution theory used for interpretation of this parameter, it is a measure for the cerebral indicator volume in the ROI.

Analyzing the PI, we found a significant dose dependence in only the temporoparietal white matter but not in the ipsilateral thalamus and lateral fissure; there were no side differences for all regions and doses. This effect could be explained by a nonlinear relationship between the high microbubble concentration in the tissue with higher capillary or artery density (thalamus/lateral fissure) and a PI in the IBS measured by the ultrasound system.10

For comparison of the washout curves of the thalamus at each side with ipsilateral and contralateral insonation, we found significant differences, indicating a significant decrease in signal intensity in investigations of similar structures at different insonation depths. Because of this significant depth dependence of the enhancing effect after UCA injection, a quantitative analysis of brain perfusion seems to be impossible through analysis of washout curves without a method for correction of this effect.

For qualitative visualization of brain perfusion, gray-scale imaging seems an appropriate imaging mode because of the homogenous echo pattern of the brain obtained after UCA injection. A comparison of our data with other human studies that used gray-scale harmonic imaging for the analysis of brain perfusion2–4 indicates that Optison has a reliable and stronger contrast-enhancing effect with less depth-dependent decrease in echo enhancement.

Ultrasound examinations and Optison injections were generally well tolerated. There were only minor adverse effects in 2 subjects, and no abnormalities in physical state or blood chemical values were noted on follow-up examination. These side effects are comparable with those published for Levovist.11

In conclusion, harmonic imaging is a useful technique for visualizing brain perfusion. Harmonic gray-scale imaging with Optison showed a strong enhancement effect in the parenchyma. A quantitative analysis of perfusion seems difficult because of the depth dependence of the effect. The most robust parameter is the intensity—heart cycle AUC for harmonic gray-scale imaging.

This study indicates that it is possible to visualize and measure changes in ultrasound intensities in perfused areas of the brain through the intact skull. This observation is encouraging for further studies evaluating brain perfusion in patients with acute brain infarctions.

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References

Harmonic Imaging of the Human Brain: Visualization of Brain Perfusion With Ultrasound
Günter Seidel, Christian Algermissen, Arnd Christoph, Lars Claassen, Marion Vidal-Langwasser and Tobias Katzer

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